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PREDICTING MULTI-TARGET AND MULTI-PATHWAY MECHANISM OF PRUNETIN AGAINST OSTEOARTHRITIS USING NETWORK PHARMACOLOGY APPROACHES

Nem Kumar Jain [1]*, Shubham Anand [2], Rajendra Singh Rajput [3], Arun Singh Kushwah [4], Prabhu Dayal Arya [5], Vinod Rathore [6] and Dinesh Singh Tomar [7]

[1,2] School of Pharmacy, ITM University Gwalior, Madhya Pradesh, India.
[3,4,5,6] School of Engineering and Technology, ITM University Gwalior, Madhya Pradesh, India.
[7] School of Sciences, ITM University Gwalior, Madhya Pradesh, India.
*Corresponding Author Email: nemkumar89@gmail.com

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Abstract

Osteoarthritis (OA) is a degenerative disorder characterized by cartilage degeneration and persistent pain, leading to disability, decreased quality of life, and substantial economic burden. Although various medicines are available for osteoarthritis, exploring phytoconstituents is garnering attention due to limited side effects. This study is based on network pharmacology to explore the potential mechanism of prunetin. First, we utilized Swissatargetprediction database to get prunetin related targets and intersected with OA targets. The intersecting targets were analysed for protein-protein interaction and GO KEGG enrichment analysis. A total of 19 core targets were identified. Even more, we obtained top 10 hub genes. Among these targets EGFR, ESR1 and PPRA were the targets significantly enriched with the core pathways such as Metabolic pathways, Chemical carcinogenesis, Arachidonic acid metabolism, and Endocrine resistance pathway. Overall, our study underscores the importance of further investigating Prunetin as a potential therapeutic option for OA and provides a foundation for future research endeavours in this field.

Keywords: Prunetin, Network Pharmacology, Phytoconstituents, Flavonoids, Osteoarthritis.

1. INTRODUCTION

Osteoarthritis (OA) is a prevalent chronic articular condition characterized by cartilage degeneration and persistent pain, leading to disability, decreased quality of life, and substantial economic burden. Its global incidence is approximately 20%, although regional disparities exist, ranging from 10–17% in Europe to 16–29% in Asia, Africa, and the Middle East. Various genetic and environmental factors influence susceptibility to OA. Traditionally viewed as a degenerative cartilage disease, OA is now recognized as a multifactorial joint pathology influenced by inflammatory and metabolic factors (Jain et al., 2020; Coaccioli et al., 2022).

Although various medicines are available for osteoarthritis, exploring herbal medicines like prunetin is significant for several reasons. Herbal medicines often offer a natural alternative with potentially fewer side effects compared to synthetic drugs (Jain et al., 2022). Additionally, herbal compounds like prunetin may possess unique bioactive properties that could provide complementary or alternative therapeutic options for managing osteoarthritis (Wal et al., 2022, 2023a). Furthermore, investigating herbal remedies contributes to a broader understanding of traditional medicine practices and may unveil novel treatment avenues for complex conditions like osteoarthritis. Therefore, exploring prunetin and similar herbal medicines is valuable in diversifying treatment options and advancing holistic approaches to osteoarthritis care. Prunetin, an isoflavone compound derived from *Caulis spatholobi*, exhibits diverse pharmacological effects, including anti-cancer and anti-inflammatory properties

(Alagusundaram et al., 2023; Fan et al., 2023). Research is ongoing to elucidate its molecular mechanisms and develop derivatives with enhanced efficacy and safety.

Clinical trials are underway to evaluate Prunetin-based therapeutics, highlighting its potential integration into personalized medicine approaches. It has several pharmacological effects, such as inflammation reduction, stress reduction, and the control of proteolytic activity. Reports suggest Prunetin's anti-OA effects, attributed to its inhibiting pro-inflammatory cytokines via the NF-kB pathway. Network pharmacology, a cutting-edge approach integrating systems biology and pharmacological data, offers insights into Prunetin's therapeutic genes and signalling pathways in OA treatment. Our study aims to unravel the molecular mechanism underlying Prunetin's ethnomedicinal use for OA by integrating bioactive compounds, molecular targets, and interacting pathways. Network pharmacology is a novel strategy integrating systems biology, and pharmacological data, which improves the clinical efficacy and understanding of the side effects of drugs (He et al., 2023; Dhanoriya et al., 2024; Jain et al., 2024a). This approach is accepted as the next model of drug development, which updates the current research model of 'single drug target' into a novel model of 'drug-disease multiple targets and multiple signalling pathways' (Shi et al., 2021; Jain et al., 2024b). In the present study, network pharmacology was performed to predict the therapeutic genes and related signalling pathways of Prunetin in the treatment of Osteoarthritis (OA). Therefore, the present study aimed to generate networks to provide a rational and molecular mechanism of the ethnomedicinal use of Prunetin as anti-OA botanical by bringing the bioactive compounds, molecular targets, and interacting pathways together.

Several studies have reported the potential efficacy of prunetin in treating osteoarthritis; however, its mechanism of action remains unclear. Hence, we conducted this study to analyse its target pathways using network pharmacology, aiming to elucidate its mode of action and therapeutic potential in osteoarthritis management.

2. METHODOLOGICAL APPROACH

We utilized a range of computational tools and databases to explore and forecast the bioactive compounds, potential gene targets, and pathways implicated in the pharmacological network of Prunetin for Osteoarthritis (OA).

Prunetin-related targets were acquired from the SwissTargetPrediction database **(http://www.swisstargetprediction.ch/)** using the Canonical SMILES acquired from PubChem Database **(https://pubchem.ncbi.nlm.nih.gov/)** (Cheng et al., 2014; Jain et al., 2023). Targets with probability score >0 were selected for futher analysis with limiting species selection of "Homo sapiens". Similarly, Osteoarthritis -related gene targets were retrieved using the keywords "Osteoarthritis, major knee disorder, and joint disorder" from DisGeNET (https://www. disgenet.org/home/) public disease databases (Piñero et al., 2017). To identify the intersected Osteoarthritis -related gene targets intervened by Prunetin active ingredients, Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/) web tool was used (Jain and Baghel, 2019; Wang et al., 2021). Intersection gene targets of selected prunetin bioactive compounds and Osteoarthritis were retrieved for subsequent network analysis. The overlapping targets of Osteoarthritis and Prunetin bioactive compounds were examined using the STRING v11.5 (https://string-db.org/) online database to identify

potential inter-target interactions (Baghel et al., 2016; Jain and Baghel, 2019; Szklarczyk et al., 2019). Official gene names of the shared targets were queried in the database with the "H. sapiens" setting, utilizing a high confidence score threshold of 0.40 to enhance data reliability. The resulting protein-protein interaction (PPI) network was exported in TSV and PNG formats and subsequently imported into Cytoscape v3.10.1 for visualization and further topological analysis. The network analyzer tool in Cytoscape v3.10.1 was employed to collect node and edge information, while the CytoNCA plug-in was utilized to identify core targets based on degree centrality. The top 10 targets were also determined using the CytoHubba plug-in, employing the degree technique among the 10 topological parameters (Shannon et al., 2003; Jain and Tailang, 2023; Wal et al., 2024). To comprehend the mechanism of Prunetin bioactive compounds in treating Osteoarthritis, the identified Osteoarthritis core targets were analyzed using the DAVID 2021 functional annotation tool (https://david.ncifcrf.gov/tools.jsp) for Gene Ontology (GO) analysis and KEGG pathway enrichment analysis (Jiao et al., 2012; Pathak et al., 2015; Wal et al., 2023b).

Official gene symbols of the core targets were inputted with "H. sapiens" as the selected species. The analyses yielded the significant GO biological processes (BP), molecular functions (MF), and cellular component (CC) terms, as well as the KEGG pathways. Data were sorted by applying filters for gene count from largest to smallest, with a significance threshold of p-value <0.05. The GO terms and KEGG pathways were visualized using SRPlot **(https://www.bioinformatics.com.cn/en)** (Dubey et al., 2014; Nelson et al., 2023). Utilizing Cytoscape v3.10.1, a Prunetin-Target-Pathway network was constructed based on the PPI network and KEGG analysis. This network incorporates Prunetin, hub gene targets, and the top 5 KEGG pathways. Nodes within the network represent bioactive compounds, targets, and pathways, while edges signify the interactions among these elements.

3. RESULTS AND DISCUSSION

3.1 Predicting Prunetin's therapeutic targets

Screening the SwissTargetPrediction database identified 48 Prunetin-related gene targets. Concurrently, 1827 targets associated with Osteoarthritis (OA) were sourced from the DisGeNET database. Upon matching these disease targets with Prunetin targets, 19 overlapping genes emerged as potential targets for the therapeutic effect of Prunetin against OA (**Fig. 1**).

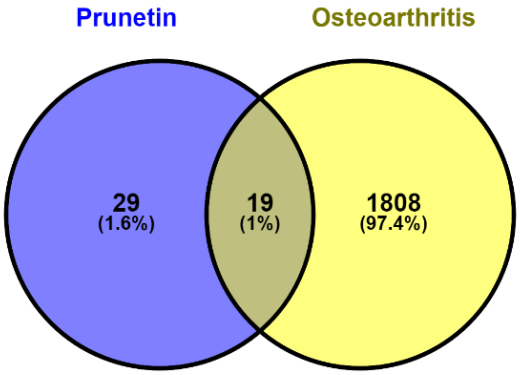

Fig 1: Screening of Potential overlapping targets between prunetin and osteoarthritis

3.2. PPI network analysis

The Protein-Protein Interaction (PPI) network comprising 19 overlapping genes was constructed using STRING and visualized through Cytoscape software. The network comprised 19 nodes and 36 edges, with an average local clustering coefficient of 0.597 and an average node degree of 3.79 (**Fig. 2**). Subsequently, visual topological analysis was conducted using the CytoNCA application, focusing on "Degree " centrality. Among the 19 core targets, the top 10 hub genes (**Fig. 3**) were determined to be ESR2: Estrogen Receptor 2, ESR1: Estrogen Receptor 1, EGFR: Epidermal Growth Factor Receptor, IL2: Interleukin 2, ESRRB: Estrogen-Related Receptor Beta, CYP19A1: Cytochrome P450 Family 19 Subfamily A Member 1, PTGS1: Prostaglandin-Endoperoxide Synthase 1, MIF: Macrophage Migration Inhibitory Factor, ESRRA: Estrogen-Related Receptor Alpha, PPARA: Peroxisome Proliferator-Activated Receptor Alpha. Notably, targets such as PTGS1, IL2 and MIF were identified as inflammation-related genes, suggesting that the inflammatory response may be a primary target for Prunetin in the treatment of Osteoarthritis (OA).

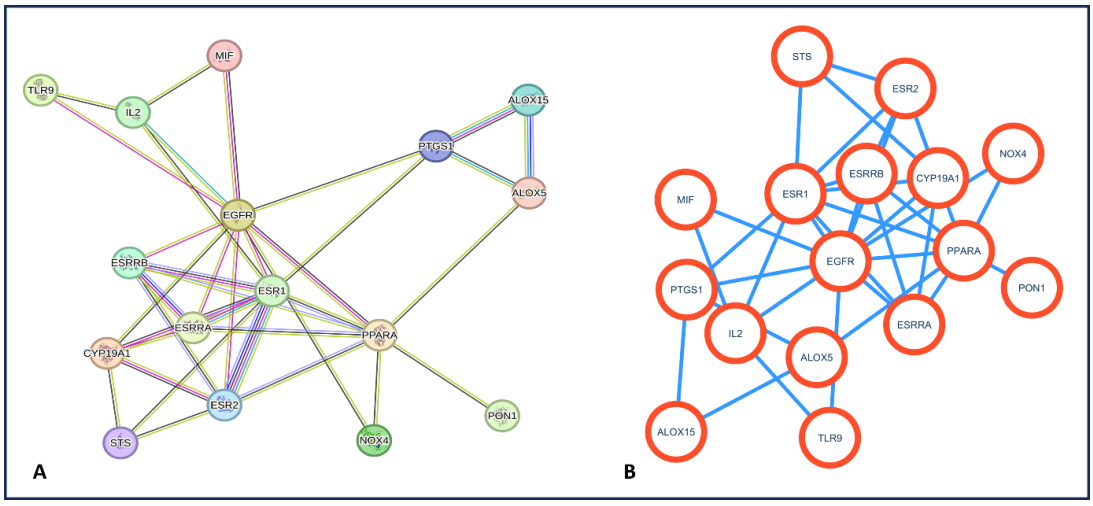

Fig 2: Protein-Protein Interaction Network: (A) STRING network, (B) Cytoscape network

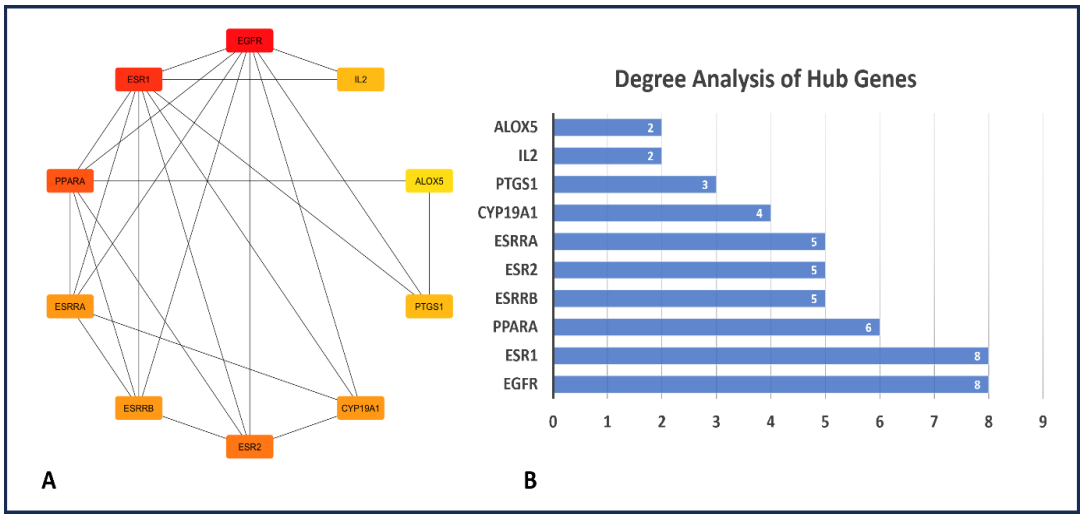

Fig 3: Hub gene targets (A) Top 10 hub gene targets, (B) Bar chart depicting degree distribution of hub gene targets

3.3. GO and KEGG pathway enrichment analysis

The 48 Osteoarthritis core targets underwent KEGG pathway and GO annotation analysis using the DAVID 2021 functional annotation tool to elucidate the molecular mechanism of prunetin in treating Osteoarthritis. Following analysis, a total of 129 significantly enriched ($p < 0.05$) GO terms were identified, encompassing 15 Biological Process (BP), 06 Cellular Component (CC), and 16 Molecular Function (MF) terms, based on the p-value. The top 5 terms for each BP, CC, and MF were selected to generate a bubble plots as shown in **Fig. 4**. Top 5 BP terms are transcription initiation from RNA polymerase II promoter, positive regulation of transcription from RNA polymerase II promoter, positive regulation of transcription, DNA-templated, inflammatory response, cytoplasm, and CC terms are GO:0005737 cytoplasm, GO:0005654 nucleoplasm, GO:0005789 endoplasmic reticulum membrane, GO:0043231 intracellular membrane-bounded organelle, GO:0000785chromatin. The most significant MF terms identified were GO:0008270 zinc ion binding, GO:0003707 steroid hormone receptor activity, GO:0004879 RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding, GO:0043565 sequence-specific DNA binding, GO:0003700 transcription factor activity, sequence-specific DNA binding. Similarly top 5 KEGG pathway were identified (**Fig. 5**). Metabolic pathways (hsa01100), Chemical carcinogenesis - receptor activation (hsa05207), Pathways in cancer (hsa05200), Arachidonic acid metabolism (hsa00590) and Endocrine resistance (hsa01522).

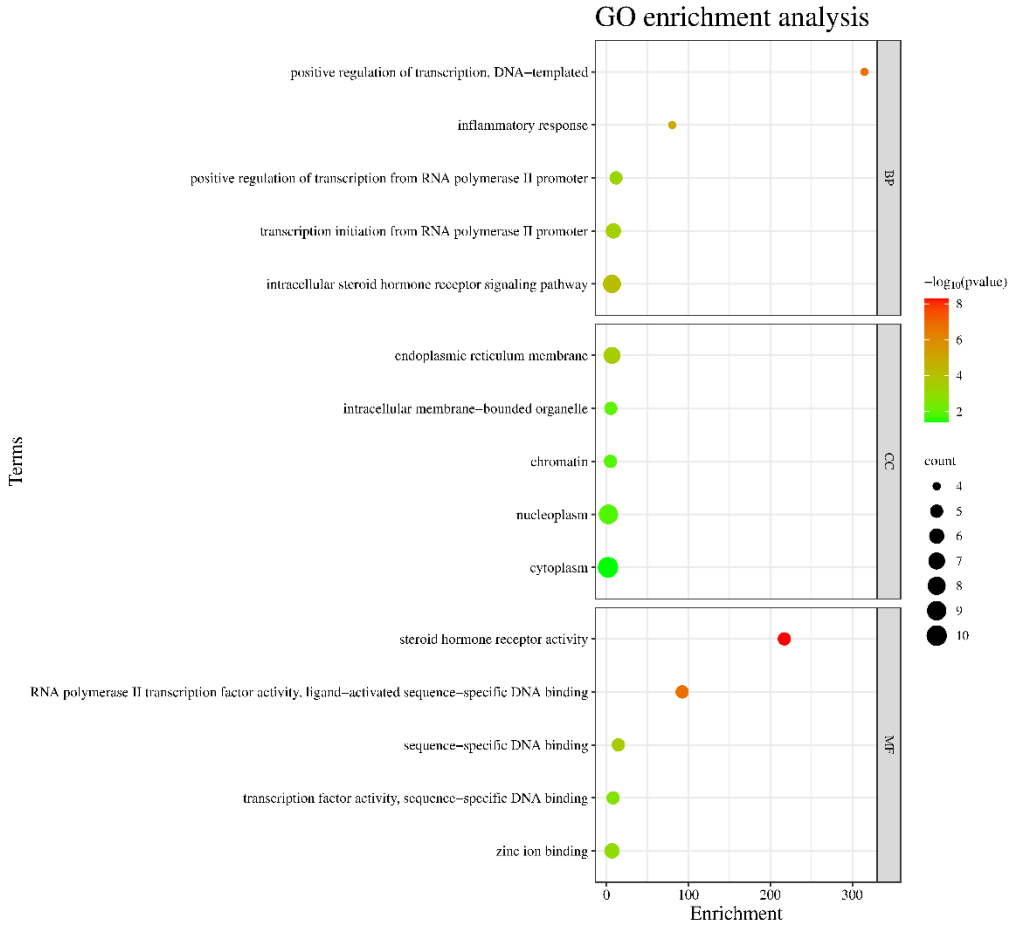

Fig 4: Gene ontology enrichment analysis

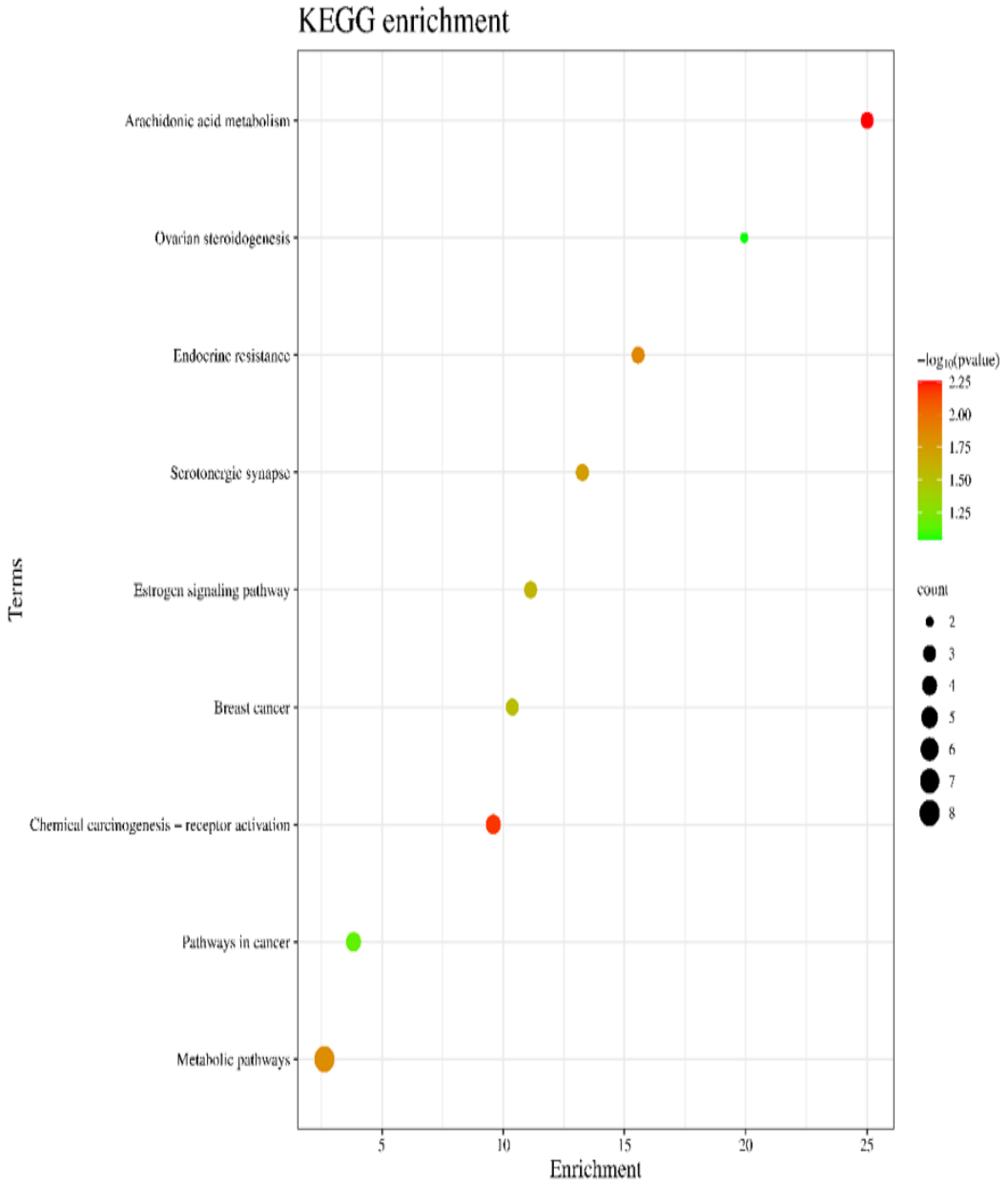

Fig 5: KEGG Pathway enrichment analysis

3.4 Prunetin-Target-Pathway network construction

Using Cytoscape v3.10.1, a visual bioactive-target-pathway network was constructed to analyze the interaction among bioactive compounds, gene targets, and significantly enriched KEGG pathways. The network comprised 26 nodes, including prunetin and five KEGG pathways, interconnected by 26 edges (**Fig. 6**).

Degree analysis highlighted certain pathways with higher degrees, notably Metabolic pathways, Chemical carcinogenesis, Arachidonic acid metabolism, and Endocrine resistance pathway. This suggests that Prunetin bioactive compounds may influence the pathogenesis of Osteoarthritis (OA) by modulating these targets enriched in the identified pathways.

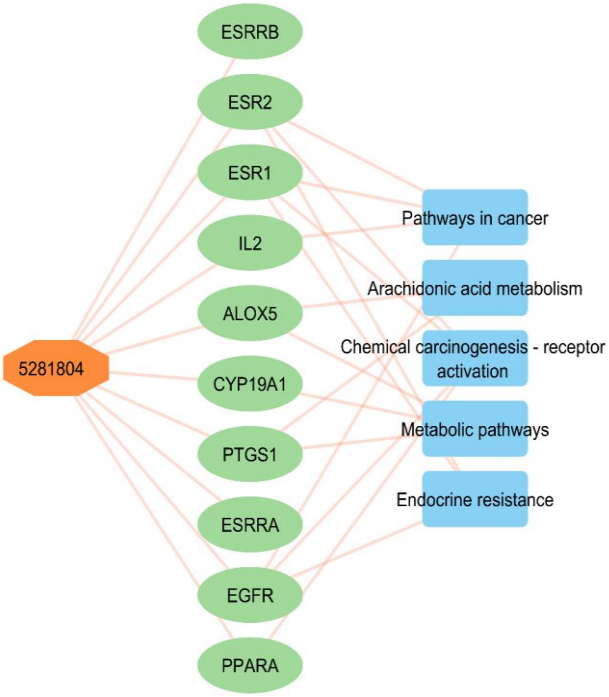

Fig 6: Prunetin- target-pathway network construction

4. CONCLUSION AND FUTURE PERSPECTIVES

The study presented here explores the potential of Prunetin, as a therapeutic option for Osteoarthritis (OA) through a comprehensive network pharmacology approach. OA is a prevalent chronic articular condition characterized by cartilage degeneration and persistent pain, leading to disability and decreased quality of life. Despite the availability of various medications, there is a growing interest in herbal medicines like Prunetin due to their potential as natural alternatives with fewer side effects. Results indicate the identification of potential target genes for Prunetin in OA treatment, along with the construction of botanical-bioactive-target networks and protein-protein interaction networks. Key genes and pathways implicated in OA pathogenesis, particularly those related to inflammation, are highlighted. Gene ontology and pathway enrichment analyses provide further understanding of Prunetin's molecular mechanisms in OA. Interpreting the findings, we underscore Prunetin's promise as anti-OA therapeutic agent. Our findings reveal that Prunetin may exert its effects through multiple pathways, including inflammatory responses, metabolic pathways and core targets identified in our study, such as Estrogen Receptors, Interleukins, and Peroxisome Proliferator-Activated Receptors, suggest a complex interplay of molecular pathways contributing to Prunetin's therapeutic effects. However, challenges such as limited clinical evidence and bioavailability hinder its translation into clinical practice. Addressing these hurdles and validating the network pharmacology findings through experimental studies are imperative for realizing Prunetin's therapeutic potential. In conclusion, our study highlights the potential of Prunetin as a therapeutic agent for Osteoarthritis (OA) through a comprehensive network pharmacology approach. By elucidating the bioactive compounds, target genes, and pathways involved, we provide valuable insights into the molecular mechanisms underlying Prunetin's efficacy in OA treatment. Overall, our study underscores the importance of further investigating Prunetin as a potential therapeutic

option for Osteoarthritis (OA) and provides a foundation for future research endeavours in this field.

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Data availability statement

The datasets presented in this study can be found in online repositories. The sources of data used in this study include publicly available databases such as PubChem, SwissTargetPrediction, DisGeNET, and STRING, Access to these databases is subject to their respective terms of use and availability.

References

1) Alagusundaram, M., Jain, N. K., Begum, M. Y., and Parameswari, S. A. (2023). Development and Characterization of Gel-Based Buccoadhesive Bilayer Formulation of Nifedipine. *Gels (Basel, Switzerland)* 9, 688. doi: https://doi.org/10.3390/gels9090688.

2) Baghel, K. S., Tewari, B. N., Shrivastava, R., Malik, S. A., Lone, M. U.-D., Jain, N. K., et al. (2016). Macrophages promote matrix protrusive and invasive function of breast cancer cells via MIP-1β dependent upregulation of MYO3A gene in breast cancer cells. *Oncoimmunology* 5, e1196299. doi: 10.1080/2162402X.2016.1196299.

3) Cheng, T., Pan, Y., Hao, M., Wang, Y., and Bryant, S. H. (2014). PubChem applications in drug discovery: a bibliometric analysis. *Drug Discov. Today* 19, 1751–1756. doi: 10.1016/j.drudis.2014.08.008.

4) Coaccioli, S., Sarzi-Puttini, P., Zis, P., Rinonapoli, G., and Varrassi, G. (2022). Osteoarthritis: New Insight on Its Pathophysiology. *J. Clin. Med.* 11. doi: 10.3390/jcm11206013.

5) Dhanoriya, D., Jain, N. K., Dhurandhar, Y., Keshri, P., Tomar, S., Kurmi, S., et al. (2024). In-Vitro Anti-Urolithiasis Activity of the Hibiscus Rosa-Sinensis L. Leaves Hydro-Alcoholic Extract on Calcium Oxalate Crystallization. *Community Pract.* 21, 1101–1112.

6) Dubey, P., Shrivastava, R., Tripathi, C., Jain, N. K., Tewari, B. N., Lone, M.-U.-D., et al. (2014). Cyclooxygenase-2 inhibition attenuates hypoxic cancer cells induced m2-polarization of macrophages. *Cell. Mol. Biol. (Noisy-le-grand).* 60, 10–15.

7) Fan, L., Shi, J., and Yang, X. (2023). Prunetin alleviates dextran sulfate sodium-induced colitis via the regulation of inflammatory response based on network pharmacology and experimental evidence. *All Life* 16, 2164359. doi: 10.1080/26895293.2022.2164359.

8) He, J., Han, D., Jia, C., Xie, J., Zhu, F., Wei, J., et al. (2023). Integrating Network Pharmacology, Molecular Docking and Pharmacological Evaluation for Exploring the Polyrhachis vicina Rogers in Ameliorating Depression. *Drug Des. Devel. Ther.* 17, 717–735. doi: 10.2147/DDDT.S399183.

9) Jain, N. K., and Baghel, K. S. (2019). Selective cyclooxygenase-2 inhibitor etoricoxib attenuated hypoxic cancer milieu induced m2-polarization of macrophages and acquisition of pro-angiogenic and pro-invasive attributes. *Res. J. Pharm. Technol.* 12, 5871–5877.

10) Jain, N. K., Roy, R., Pathan, H. K., Sharma, A., Ghosh, S., and Kumar, S. (2020). Formulation and evaluation of polyherbal aqueous gel from Psidium guajava, Piper betel and Glycerrhiza glabra extract for mouth ulcer treatment. *Res. J. Pharmacogn. Phytochem.* 12, 145–148.

11) Jain, N. K., and Tailang, M. (2023). Green synthesis of zinc oxide nanoparticles and their biomedical applications in cancer treatment: current status and future perspectives. *Appl. Nanosci.* 13, 6605–6629. doi: 10.1007/s13204-023-02946-8.

12) Jain, N. K., Tailang, M., Chandrasekaran, B., Khazaleh, N., Thangavel, N., Makeen, H. A., et al. (2024a). Integrating network pharmacology with molecular docking to rationalize the ethnomedicinal use of Alchornea laxiflora (Benth.) Pax & K. Hoffm. for efficient treatment of depression. *Front. Pharmacol.* 15, 1290398. doi: 10.3389/fphar.2024.1290398.

13) Jain, N. K., Tailang, M., Jain, H. K., Chandrasekaran, B., Sahoo, B. M., Subramanian, A., et al. (2023). Therapeutic implications of current Janus kinase inhibitors as anti-COVID agents: A review. *Front. Pharmacol.* 14, 1135145. doi: 10.3389/fphar.2023.1135145.

14) Jain, N. K., Tailang, M., Kumar, S., Chandrasekaran, B., Alghazwani, Y., Chandramoorthy, H. C., et al. (2022). Appraising the therapeutical potentials of Alchornea laxiflora (Benth.) Pax & K. Hoffm., an underexplored medicinal herb: A systematic review. *Front. Pharmacol.* 13, 958453. doi: 10.3389/fphar.2022.958453.

15) Jain, N. K., Tailang, M., Thangavel, N., Makeen, H. A., Albratty, M., Najmi, A., et al. (2024b). A comprehensive overview of selective and novel fibroblast growth factor receptor inhibitors as a potential anticancer modality. *Acta Pharm.* 74, 1–36. doi: 10.2478/acph-2024-0005.

16) Jiao, X., Sherman, B. T., Huang, D. W., Stephens, R., Baseler, M. W., Lane, H. C., et al. (2012). DAVID-WS: a stateful web service to facilitate gene/protein list analysis. *Bioinformatics* 28, 1805–1806. doi: 10.1093/bioinformatics/bts251.

17) Nelson, V. K., Nuli, M. V., Mastanaiah, J., Saleem T. S., M., Birudala, G., Jamous, Y. F., et al. (2023). Reactive oxygen species mediated apoptotic death of colon cancer cells: therapeutic potential of plant derived alkaloids. *Front. Endocrinol. (Lausanne).* 14, 1201198. Available at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1201198.

18) Pathak, M. K., Jha, V., Jain, N. K., Shrivastava, N., Yadav, N., and Purukayastha, D. (2015). Review on peptidomimetics: a drug designing tool. *Am. J. Pharm. Res.* 5, 3859–3867.

19) Piñero, J., Bravo, À., Queralt-Rosinach, N., Gutiérrez-Sacristán, A., Deu-Pons, J., Centeno, E., et al. (2017). DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Res.* 45, D833–D839. doi: 10.1093/nar/gkw943.

20) Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., et al. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504. doi: 10.1101/gr.1239303.

21) Shi, Y., Chen, M., Zhao, Z., Pan, J., and Huang, S. (2021). Network Pharmacology and Molecular Docking Analyses of Mechanisms Underlying Effects of the Cyperi Rhizoma-Chuanxiong Rhizoma Herb Pair on Depression. *Evid. Based. Complement. Alternat. Med.* 2021, 5704578. doi: 10.1155/2021/5704578.

22) Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., et al. (2019). STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* 47, D607–D613. doi: 10.1093/nar/gky1131.

23) Wal, A., Wal, P., Gupta, D., Kumar, A., Sharma, P., and Jain, N. K. (2022). Natural products: a rising star for treating primary dysmenorrhea? *Tradit. Med. Res.* 7, 55. doi: 10.53388/tmr20220410001.

24) Wal, A., Wal, P., Vig, H., Jain, N. K., Rathore, S., Krishnan, K., et al. (2023a). Treatment of Parkinson's Disease: Current Treatments and Recent Therapeutic Developments. *Curr. Drug Discov. Technol.* doi: 10.2174/1570163820666230512100340.

25) Wal, P., Shukla, V., Uzzaman Khan, M. M., Gaur, K., Wal, A., and Jain, N. K. (2024). Medicinal Properties of Crotalaria burhia: A Review. *Curr. Tradit. Med.* 10, 90–100. doi: http://dx.doi.org/10.2174/2215083810666230428095559.

26) Wal, P., Vig, H., Wal, A., Rathore, S., Pandey, S. S., Jain, N. K., et al. (2023b). Role of Nutraceuticals and Physical Activity in Parkinson's Disease Risk and Lifestyle Management. *Curr. Aging Sci.* 16, 170–187. doi: http://dx.doi.org/10.2174/1874609816666230515121717.

27) Wang, Y., Zhang, Y., Wang, Y., Shu, X., Lu, C., Shao, S., et al. (2021). Using Network Pharmacology and Molecular Docking to Explore the Mechanism of Shan Ci Gu (Cremastra appendiculata) Against Non-Small Cell Lung Cancer. *Front. Chem.* 9, 682862. doi: 10.3389/fchem.2021.682862.